Participation of the Tightly-Bound (Putative Cytoskeleton-Bound) Polysomes in Translation during Germination of Dormant and Non-Dormant Cereal Caryopses

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Research was done on dormant and non-dormant barley cv. Ars caryopses and triticale cv. Grado caryopses treated and non-treated with abscisic acid (ABA). During germination higher participation of populations of so-called tightly-bound polysomes (TBP) in embryos of dormant barley caryopses was observed, as well as their high metabolic activity. In embryos of triticale caryopses of which dormancy was imposed in an artificial way by ABA (100 μM), the strongest incorporation of ^{14}C-amino acids into nascent polypeptide chains *in vivo* was found in population of TBP, as well as the highest participation among three of the studied fractions (free polysomes, membrane-bound polysomes and tightly-bound polysomes). These results may indicate the significant role of TBP (putative cytoskeleton-bound polysomes – CBP) in maintaining dormancy during imbibition of cereal caryopses.

Introduction

Harvest-ripe cereal grains may not germinate immediately. While this is advantageous in the field, protecting the crop against sprouting in the ear, it is clearly a problem in relation to malting, which depends on active germination. Glumless caryopses such as rye, triticale and wheat are especially prone to precocious germination. This phenomenon sometimes causes great world-wide losses (Derera, 1990). The mechanism of dormancy is not fully understood (Simpson, 1990; Bewley and Black, 1994).

It has recently become clear, that the initiation of dormancy involves the action of ABA (Bewley, 1997; Li and Foley, 1997). Initiation of dormancy may also depend on seed sensitivity to ABA (Weidner, 1987). Evidence that the degree of sensitivity to ABA is indeed important comes from studies on dormancy in wheat grains. Differences in susceptibility to pre-harvest sprouting occur among cultivars according to the intensity of dormancy. But though the dormancy varies, the ABA concentrations are similar (Walker-Simmons, 1987). Isolated embryos of both types (resistant and susceptible to sprouting) also germinate equally well on water. Differences show up, however, in the presence of ABA. Those of the high-dormancy cultivar respond more to ABA (Walker-Simmons, 1988; Morris *et al.,* 1989).

Major action of ABA in seeds (and other plant parts) is the regulation of gene expression, particularly the induction of several different kinds of polypeptides and the inhibition of genes for certain reserve mobilising enzymes (Dommes and Northcote, 1985; Jacobsen and Chandler, 1987; Skiver and Mundy, 1990). When ABA is acting to induce dormancy it also causes formation of a spectrum of proteins. Some of these proteins probably provoke the initiation of dormancy (Black, 1991). ABA-responsive genes are expressed more readily in embryos of dormant grains, raising the possibility that set of "dormancy proteins" exists. So far it has not been explained, what the function and mechanism of action of these proteins is.

Regulation of gene expression by ABA also occurs, at the post-transcriptional and/or the translational level (e.g. Bray, 1991). For example, osmotion, a salt-regulated gene product, acumulates in tobacco suspension cultures in response to ABA only in a low water potential environment even though the mRNA accumulates in response to

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ABA application at high and low water potentials (Singh *et al.,* 1989). This indicates that ABA requires additional signals that are only present at low water potentials to regulate translation.

Lenk *et al.* (1977) suggested that the cytoskeleton of animal cells could serve as an attachment site for a subpopulation of polysomes, the cytoskeleton-bound polysomes (CBP). The fundamental role of the cytoskeleton in subcellular transport and localization of mRNAs in animals is widely accepted (St. Johnston, 1995). Since polysomes are the site of translation and thus mediate the final step in gene expression, their subcellular location is important for the ultimate density of their products. It is assumed that in animal cells free polysomes (FP) are involved primarily in the synthesis of cytosolic proteins, membrane-bound polysomes (MBP) are involved in the synthesis of secreted and sequestrated proteins and cytoskeleton-bound polysomes (CBP) in the synthesis of cytoskeletal, virial, or stress proteins (Elder and Morré, 1978; Adams *et al.,* 1983; Hesketh and Pryme, 1991).

The objective of this research was to show the presumable role which population of the tightly-bound polysomes (putative cytoskeleton-bound polysomes) may play in translation, during germination of dormant barley caryopses and treated with ABA triticale grains.

Materials and Methods

Plant material, germination conditions and conditions of labelling

The study was conducted on barley (*Hordeum vulgare* L.) *cv.* Ars and triticale (*Triticosecale*) *cv.* Grado cultivated in an experimental field of the Warmia and Masuria University in Olsztyn. Barley caryopses in full ripeness were collected and divided into two batches. One batch was examined immediately after harvest (dormant caryopses), the other batch was kept for eight months in a laboratory at 20 °C and dry air. During eight months storage in the dry state, caryopses were completely released from dormancy. This batch (non-dormant caryopses), was also subjected to the same comparative investigations. Triticale caryopses collected in full ripeness, after releasing of (very shallow) dormancy, were treated with different concentration of ABA (0, 1, 10 and 100 μM) during germination.

The caryopses before germination were washed with tap water, placed for 3 min in a 1% solution of sodium hypochlorite and washed thoroughly with cool, redistilled sterile water. The materials were then subjected to 48 hrs germination in water (or in ABA solutions) on Petri dishes in darkness at 21–22 °C. After 48 hrs germination barley caryopses were transferred into a solution of radioactive precursors and germinated for 6 hrs. Germination was conducted in the presence of [^{14}C]-amino acid hydrolysate (3.7 MBq·ml^{-1}) or [^{3}H]-uridine (3.7 MBq·ml^{-1}) and chloroamphenicol (10 μg·ml^{-1}). At specific times, embryos (or germs) were isolated from grains, the nonincorporated precursor was carefully rinsed off, the embryo surfaces dried and then stored in liquid nitrogen until further study.

Triticale caryopses after initial 48 hrs of germination in water or in ABA solutions (1, 10 and 100 μM) were transferred into solution of [^{14}C]-amino acid hydrolysate (3.7 MBq·ml^{-1}) and chloramphenical (10 μg·ml^{-1}). Caryopses were germinated in the presence of radioactive precursors for 30 min. Embryos (or germs) were isolated from grains after definite germination time, nonincorporated precursors was carefully rinsed off, the embryo surfaces dried and three polysome fractions were extracted from this plant material.

Polysome isolation and sucrose gradient analysis of polysomes

Germinating barley or triticale embryos were homogenized in buffer A consisting of 200 mM Tris [tris(hydroxymethyl)aminomethane]-HCl, pH 8.5, 50 mM potassium acetate, 25 mM magnesium acetate (Davies *et al.,* 1972) filtered and centrifuged at 27000 x*g* for 10 min to release the free polysomes (FP) into the supernatant. The pellet was extracted sequentially in buffer A supplemented with 2% PTE (polyoxyethylene-10-tridecyl ether, a non-ionic detergent) to yield released MBP (membrane-bound polysomes). The supernatants were kept on ice and the tightly-bound polysomes retained in the pellets were solubilized in buffer U consisting of buffer A supplemented with 2% PTE, 1% DOC (sodium deoxycholate), 2 mM EGTA (ethylene glycol-bis(β-aminoethyl ethe-

r)N,N,N′,N′- tetraacetic acid) and 100 μg·ml^{-1} heparin, which is used to solubilize virtually all polysomes from any source (Abe *et. al.,* 1992). After incubation for 10 min, pellets were resuspended by vortexing using a grass rod in each tube, buffer U extracts were recentrifuged for 10 min at 27000 x*g*. All the supernatants were layered on 4 ml "pad" of 50% (w/v) sucrose in buffer B (50 mM Tris-HCl, pH 7.5; 20 mM potassium acetate, 10 mM magnesium acetate) and centrifuged for 3 h at 300000 x*g* in a Beckman 65 Ti rotor. The polysome pellets were rinsed in water and then resuspended in 1 ml buffer U. The resuspended polysomes were centrifuged at top speed (approx. 18000 x*g*) for 2 min in a microfuge prior to layering 0.2 ml aliquots of supernatant on linear 15 to 60% (w/v) sucrose gradients in buffer B. Separation of subunits, monosomes and polysomes was achieved by centrifugation at 122000 x*g* in a Beckman SW-41 rotor for 75 min. All operations were conducted at 0–4 °C. Gradients were next scanned at 254 nm on an UA-5 flow recorder (ISCO, Lincoln, NE, USA). The percentage of polysomes in the total ribosomal preparation was calculated by xeroxing the A_{254} profile, drawing the base line (obtained by scanning a blank gradient) under the profile, and then cutting out and weighing the paper (uniform in thickens) representing the areas of monosomes and polysomes of the profiles. Quantitation of the ribosomes was done assuming that the absorbance (A) of a 1% solution of ribosomes (measured in a cuvette with a 1 cm optical path at 260) equals A_{260} = 13.5 (Gualerzi and Cammarano, 1969). Radioactvity was assayed using a Beckman LS-1801 liquid scintillation counter, applying Tritosol as the scintillaton (Fricke, 1973). All experiments were done at least in triplicate.

Results and Discussion

Participation of free membrane-bound and tightly-bound (putative cytoskeleton-bound) polysomes in the regulation of barley caryopses dormancy and germination

Using conventional polysome isolation buffers were obtained two populations of polysomes, by homogenizing embryos in buffer A – to yield free polysomes (FP) and extracted from pellet in buffer A + PTE – membrane bound polysomes (MBP) (Davies *et al.,* 1972; Larkins and Davies, 1975). It appeared however, that some polysomes still retained in pellet, the so-colled tightly-bound polysomes (TBP) (Davies *et al.,* 1991). This population of polysomes may be released by using buffer U (Abe *et al.,* 1992) (Figs 1 and 2), which is used to solubilize virtually all polysomes from any source.

Animals have been shown to possess three populations of cytoplasmic polysomes, free (FP), membrane-bound (MBP) and cytoskeleton-bound (CBP) (Nielson *et al.*, 1983). This led us to speculate that plants also might contain a population of CBP, but that had been overlooked. Recently, we got much more proofs for the existence of the cytoskeleton-bund-polysomes in plants (e.g. Davies *et al.,* 1988).

Pea stem tissue extracted in buffer A yields over 50% of polysomes in the supernatant – the FP (Davies *et al.,* 1972), about 30% released by nonionic detergent – the MBP (Larkins and Davies, 1975) and about 15% in the detergent insoluble pellet – the TBP (Abe and Davies, 1985). Proportions of polysome populations isolated from barley embryos by using buffer A – releasing FP was similar – about 60%, both in dormant and nondormant caryopses (Fig. 1a). More than 20% in both cases made fractions of polysomes isolated with the help of buffer A + PTE which releases MBP. A little greater differences were observed after reextraction of pellet by using the buffer U, which releases TBP (putative CBP). Proportion of this population of polysomes isolated from embryos of dormant caryopses (after 48 hrs imbibition) was about 16%, whereas after its releasing from dormancy about 10% (Fig. 1a). The research also included synthesis of polysomal RNA and proteins *in vivo* (Figs 2a and 2b). In all cases the highest incorporation of radioactive precursors was observed in fraction TBP (putative CBP). It indicates the high metabolic activity of this fraction of polysomes.

It should be emphasized that polysome formation of all mentioned polysome populations was strongly inhibited in embryos of dormant caryopses during imbibition (Figs 2a and 2b). In many cases correlation was demonstrated between embryo or cotyledon growth, polysome formation and their total capacity for protein synthesis (e.g. Gwózdź and Deckert, 1989).

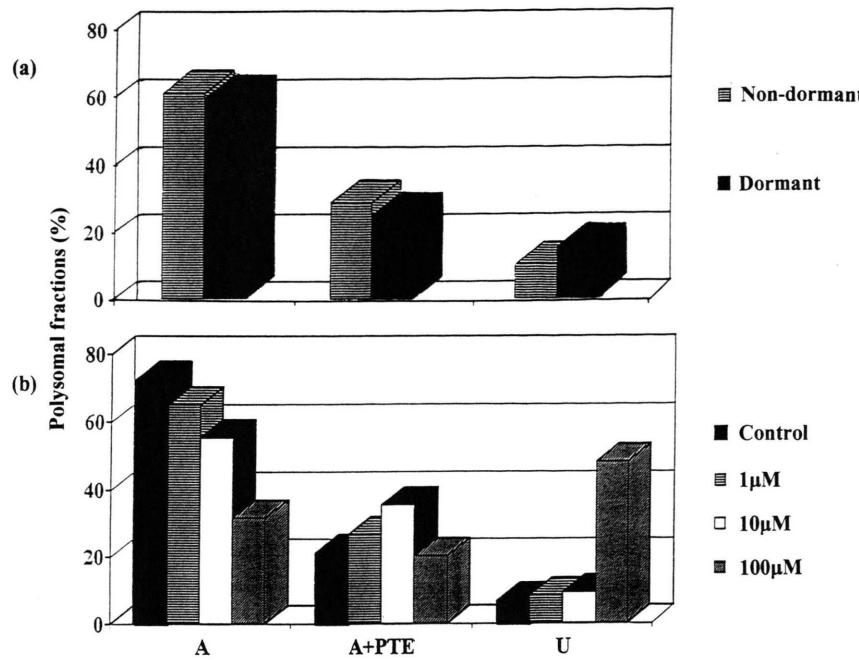

Fig. 1 (a) and (b). The percentage of the polysomal populations (FP, MBP and TBP) in the total ribosomal material, after sequential extraction of barley or triticale embryos in different buffers. Three polysome fractions were extracted from this plant material (FP-free polysomes, MBP-membrane-bound polysomes and TBP-tightly-bound polysomes). Initially the cereal tissue was homogenized in buffer A (200 mM Tris-HCl, pH 8.5, 50 mM KOAc, 25 mM MgOAc) – to yield FP. The pellet was extracted sequentially in buffer A + PTE (polyoxyethylene-10-tridecyl ether) – to release MBP. Polysomes retained in pellet were solubilized in buffer U (buffer A supplemented with 2% PTE, 1% DOC, 2 mM EGTA and 100 $\mu g \cdot ml^{-1}$ heparin) – to yield the TBP (putative CBP). (a) Barley embryos (or germs) were isolated from dormant and non-dormant grains after 48 hrs of germination. (b) Triticale grains were germinated in water (control) and in the presence of abscisic acid in following concentrations: 1 μM, 10 μM and 100 μM. After 48 hrs of germination the embryos (or germs) were isolated from the caryopses.

Polysomes formation and translation activity of free, membrane-bound and tightly-bound (putative cytoskeleton-bound) polysomes in embryos of germinating triticale caryopses in the presence of abscisic acid

It has been well documented that abscisic acid (ABA) effects many physiological processes, including seed development, dormancy and germination (Bewely and Black, 1984). In barley aleurone layers ABA suppresses the expression of genes encoding GA induced protein i.e. α-amylase and β-gluconase (Higgins *et al.*, 1982) and also increases the levels of the ABA-induced proteins (Jacobsen and Chandler, 1987; Lin and Ho, 1988).

In our research ABA inhibited ability of triticale caryopses to germinate, proportionally to its concentration. After 48 hrs per cent of germination in particular batches was as follows: control – 95.2 ± 1.76; 1 μM ABA solution – 86.4 ± 4.32; 10 μM ABA – 73.4 ± 4.23 and 100 μM ABA – 54.4 ± 4.56. ABA also influenced accumulation of dry and fresh weight of embryos during germination of caryopses (data not presented). Definitely the highest inhibition of germination processes was observed in embryos of caryopses germinating in ABA solution at 100 μM concentration. Medium dry weight of embryo (during 48 hrs of germination) increased 2.91 mg in the control and only 0.32 mg in the sample treated with the highest concentration of ABA.

The highest participation of free polysome populations (FP), isolated by buffer A, was observed both in control sample and in samples treated with ABA at 1 and 10 μM concentration. It was as follows: 73.5, 65.1 and 55.4% (Fig. 1b). In these three groups was observed the increase of participation of membrane-bound polysome populations iso-

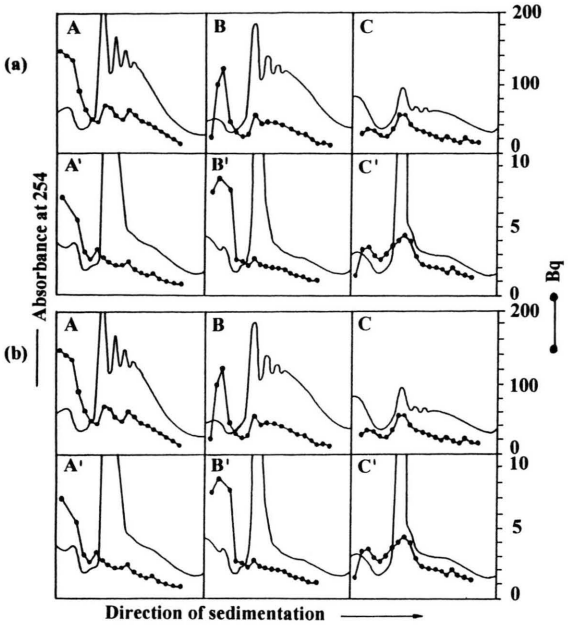

Fig. 2 (a) and (b). Sedimentation in sucrose gradients of polysomes of germinating non-dormant (A, B, C) and dormant (A′, B′, C′) barley caryopses. After initial 48 hrs of germination in water, caryopses were transferred into solution of [^{3}H]-uridine (a) or [^{14}C]-amino acids (b) and were labelled for 6 hrs. Embryos (or germs) were isolated from grains after 48 + 6 hrs germination and three polysome fractions were extracted from this plant material (FP-free polysomes, MBP-membrane-bound polysomes and TBP-tightly-bound polysomes). Initially the cereal tissue was homogenised in buffer A (200 mM Tris-HCl, pH 8.5, 50 mM KOAc, 25 mM MgOAc) – to yield FP (A, A′). The pellet was extracted sequentially in buffer A + PTE (polyoxyethylene-10-tridecyl ether) – to release MBP (B, B′). Polysomes retained in pellet were solubilized in buffer U (buffer A supplemented with 2% PTE, 1% DOC, 2 mM EGTA and 100 μg·ml^{-1} heparin) – to yield the TBP – putative CBP (C, C′).

lated by buffer A + PTE proportionally to increase of concentrations of abscisic acid. It was in the control group 21%, and in samples treated with ABA at 1 and 10 μM concentrations respectively: 26.6 and 35.4%. Participation of TBP (isolated by buffer U) was in these groups very low, no higher than 10%. Other dependence was observed however, in group of caryopses exposed to germination in 100 μM concentration of ABA. In embryos of these caryopses (in which processes of germination were inhibited almost completely), the highest participation had population TBP – 48.2%, lower population FP – 31.6% and the lowest population MBP – 20.2% (Fig. 1b).

In next part was defined the percentage of polysomes in the total ribosomal materials (sub-units, monosomes and polysomes) in particular populations of FP, MBP and TBP, extracted from embryos tissue, after 48 hrs of triticale caryopses germination (Table I). The highest participation of polysomes in all studied populations was found in embryo tissue from the control group. Polysomes made up here 81.67% of total ribosomal material extracted by buffer A (FP), 78.26% – by buffer A + PTE (MBP) and 56.45% by buffer U (TBP). Much smaller participation of polysomes was observed in ribosomal fractions isolated from embryos of caryopses treated with ABA. By applying the highest concentration of ABA (100 μM), the participation was 31% in fraction FP, 46.91 in fraction MBP and 19.64% in fraction TBP (Table I).

In the opinion of Dommes and Van de Walle (1990) and You *et al.* (1992) during short 20–30 min of incubation with labelled amino acids, the vast majority of polysome associated label is in nascent protein. Incorporation of [^{14}C]-amino acids into nascent polypeptide chains in control sample (after 48 hrs of germination of caryopses + 30 min of incubation with precursors) was as follows: in fraction isolated by buffer A – 143 (FP), by buffer A + PTE – 700 (MBP), and by buffer U – (TBP) 1421 Bq·mg^{-1} of polysomes, whereas in the sample treated with ABA (concentration – 100 μM) was 16, 136 and 204 Bq·mg^{-1} of polysomes respectively. The highest incorporation of precursors into newly formed proteins was observed in both examined samples in the last fraction of polysomes, isolated with the help of the buffer U (TBP). In the sample treated with ABA (100 μM), the activity of this population of polysomes in forming new proteins in relation to FP was about 13 times higher. The highest incorporation of [^{3}H]-uridine into polysomal RNA in the TBP was also found during precocious germination of triticale caryopses (Weidner and Łukaszewicz, 1977). It should be emphasised that protein synthesis with the participation of all mentioned polysome populations was strongly inhibited in embryos of germinating caryopses in the presence of ABA (Table I). These results are in agreement with our earlier research conducted on embryos of germinating wheat caryopses (Weidner *et al.*, 1991). We found that the

Table I. The percentage of polysomes in the total ribosomal materials (sub-units monosomes and polysomes) of each of examined polysome populations and the incorporation [^{14}C]-amino acids into polysome fractions of triticale embryos during germination of caryopses. After initial 48 hrs of germination in water or in ABA solutions caryopses were transferred into solution of radioactive precursors and were labelled for 30 min. Embryos (or germs) were isolated from grains after definite germination time and three polysome populations (FP-free polysomes, MBP-membrane-bound polysomes, TBP-tightly-bound polysomes) were extracted from this plant material. Initially the cereal tissue was homogenized in buffer A (200 mM Tris-HCl, pH 8.5, 50 mM KOAc, 25 mM MgOAc) – to yield FP. The pellet was extracted sequentially in buffer A + PTE (polyoxyethylene-10-tridecyl ether) – to release MBP. Polysomes retained in pellet were solubilized in buffer U (buffer A supplemented with 2% PTE, 1% DOC, 2 mM EGTA and 100 $\mu g \cdot ml^{-1}$ heparin) – to yield the TBP (putative CBP).

Treatment	Buffer					
	A		A + PTE		U	
	polysomes %	Bq · mg^{-1} polysomes	polysomes %	Bq · mg^{-1} polysomes	polysomes %	Bq · mg^{-1} polysomes
Control	81.67	143	78.26	700	56.45	1421
1 μM ABA	72.67	–	71.30	–	50.76	–
10 μM ABA	64.13	–	64.70	–	45.79	–
100 μM ABA	31.00	16	46.91	136	19.64	204

[^{3}H]-leucine incorporation into protein *in vitro*, by free and membrane-bound polysomes from embryos incubated with ABA (100 μM concentration), was lower in all stages of germination. The synthesis of a 28 kDa peptide was strongly inhibited by ABA, whereas the appearance of others was delayed.

When dormancy was imposed by 100 μM ABA the highest participation of population of tightly-bound polysomes (TBP) and the highest incorporation of [^{14}C]-amino acids into nascent polypeptide chains in embryos of triticale caryopses was observed. This finding and also a higher participation of this fraction and their high metabolic activity during germination of dormant barley caryopses, may indicate the significant role of this subpopulation of polysomes (and proteins synthesized by them) in dormancy of cereal caryopses.

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